MICROSCOPY RESEARCH AND TECHNIQUE 57:120–129 (2002)

Adrenomedullin Has Multiple Roles in Disease Stress: Development and Remission of the Inflammatory Response

TED H. ELSASSER* AND STAS KAHL
Growth Biology Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland

KEY WORDS adrenomedullin; inflammation; homeostasis; disease

ABSTRACT The upregulation of adrenomedullin (AM) gene expression and increases in systemic circulatory as well as localized tissue AM concentrations is well coordinated with the onset and progression of trauma, infection, and sepsis. As such, the coordinated change in AM suggests a key role for this peptide in the inflammatory response. By clinical definition, the process of inflammation constitutes an orchestrated cascade of localized tissue and systemic responses to immunological challenges. Classical responses to the onset of disease stresses are manifested in the timely elaboration of humoral, blood-borne signal effectors (such as adrenocortical and locally produced tissue hormones, immune cytokines, and inorganic signals such as nitric oxide) as well as patterned migration and infiltration of circulating bone marrow-derived cells (mononuclear cells such as monocyte-macrophages and polymorphonuclear cells like neutrophils) largely associated with or delivered through the vascular system. The body's attempts to combat acute infection to restore homeostatic equilibrium are further compromised by underlying disease situations. Atherosclerosis, diabetes, and cardiovascular disease, as well as nutritional metabolic derangements and persistent subclinical infection perturb the regulatory feedback loops necessary for proper control of response effectors like hormones and cytokines. When imbalances occur, tissue necrosis can ensue as driven by free radical damage to cell components. A true appreciation of the inflammatory response can only be grasped through an integrative approach in which the relationship between the different physiological systems is viewed in terms of a changing, dynamic interaction. In essence, the inflammatory response can be thought of in three phases: a period of severity assessment, a period of remediation, and a period of homeostatic restoration. Indeed, AM has differential effects on cellular metabolism, immune function, endocrine function, and cardiovascular function. This peptide appears to play a pivotal role in both reprioritizing the biological needs of tissues and organs during the three phases of inflammatory response as well as a role in restoring homeostatic equilibrium to the body. *Microsc. Res. Tech. 57:120–129, 2002.* Published 2002 Wiley-Liss, Inc.†

INTRODUCTION

"Normal" body function is characteristically defined in terms of quantifiable (and operational) upper and lower limits of some biomarker (a plasma enzyme concentration, heart rate, a cation or anion concentration, body temperature, etc.) usually bracketed within relative degrees of patterned fluctuation (in engineering terms, "hysteresis"). This range of normal response results from the regulatory feedback loops in organ systems within which reside mechanisms to monitor and modulate the internal environment of the body. Classically, these mechanisms involve some form of receptor to bind the monitored modality, a signal transduction response to interpret and integrate combined signals, and a response (effector) that acts to change some aspect (concentration, etc) of the monitored component. When there is controlled balance in the body between physiological systems, the body is deemed to be in homeostatic equilibrium. In these periods of equilibrium, biological priorities among processes and tissues is well defined and, in fact, frequently redefined to accommodate the changing demands of the normal processes of growth and development, aging, reproductive and lactational status, nutritional status, etc. In the face of perceived threats to health, as monitored by the immune system, the body secretes hormones and cytokines to counter the threat. At any given time the metabolic priorities of cells and tissues reflect the higher priority of species survival and as such largely coincide with the need to escape from or combat external (trauma) and internal (immunological) threats.

Recently, we extended a provisional hypothesis originally set forth by Sir John Hammond (1952), wherein a hierarchy of metabolic priorities between tissues was described. In the original presentation, tissue priority was associated with tissue metabolic rate. Thus, brain and neural tissues were ascribed highest priorities and access to nutrients and vascular flow. Adipose tissues were assigned the lowest priorities, especially in times of calorie excess in the diet. Our extension of this model attempted to impart a mechanistic level of control of

*Correspondence to: Ted H. Elsasser, Growth Biology Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD. E-mail: elsasser@anri.barc.usda.gov

Received 7 June 2001; accepted in revised form 8 August 2001
DOI 10.1002/jemt.10058
Published online in Wiley InterScience (www.interscience.wiley.com).

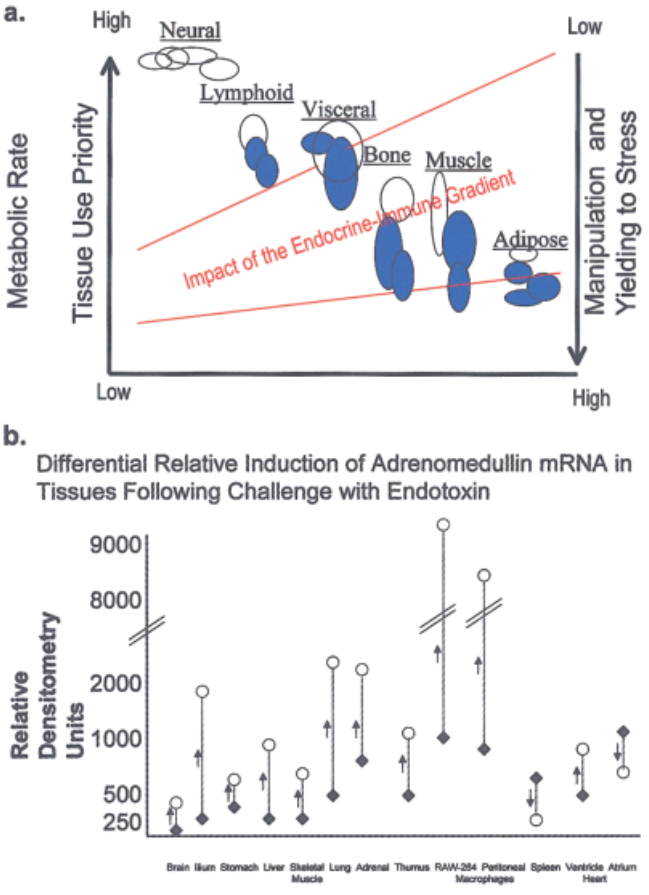

Fig. 1. Cell and tissue responses to immune challenge are the culmination of cellular integration of numerous endocrine and immune system secretions which regulate metabolism (**a**). Depicted as the "endocrine-immune gradient (Elsasser et al., 2000a), the figure demonstrates that there exists among organs in different biological systems (different organs are depicted by various ellipsoid shapes) and between organs in the same system a hierarchy through which survival is achieved (open circle is highest priority for use access). Tissues of lowest metabolic need are last to be conserved in times of stress and first to be mobilized to provide additional nutrient reserves for processes of "higher" biological need. Adrenomedullin is a newly added component of the endocrine-immune gradient with functional attributes that cross over system boundaries to interact in immune, endocrine, and metabolic regulation of tissue function. AM is differentially upregulated by endotoxin (as a model of immune stress) in many tissues (**b**). Note that even between different cells and organs of the immune system the magnitude and directional change in AM mRNA abundance before and after endotoxin challenge varies greatly, thus giving a further measure of site-specific tissue response control to the overall inflammatory process. (Adapted from Shoji et al., 1995; Kubo et al., 1998b; Zaks-Zilberman et al., 1998.)

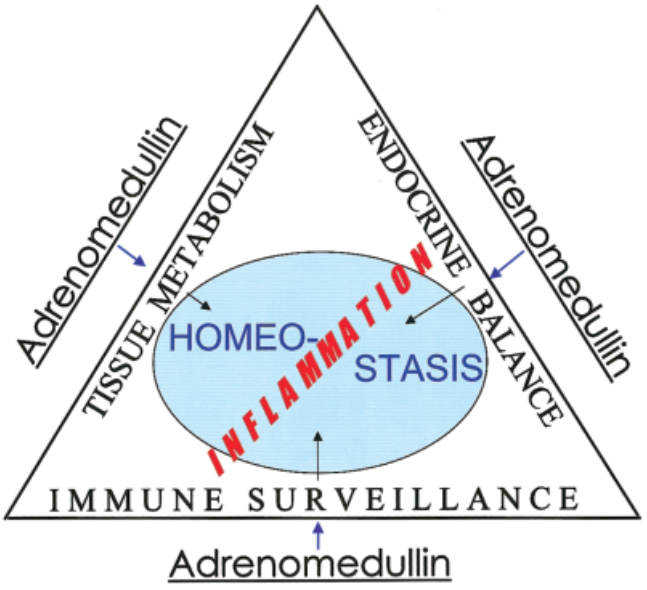

Fig. 2. Trilogy of homeostasis. Adrenomedullin possesses numerous biological qualities and functions that make it a key integrator of the cellular responses to inflammation which assist in restoring homeostasis.

how these priorities were established and controlled. We coined the term "endocrine-immune gradient" (Elsasser et al., 2000b) to provide a mechanistic framework upon which a dynamic model of endocrine and immune system interactions could be assembled to explain how tissue beds could be affected differentially and with varying degrees of severity to the perturbations of disease stress. In this scheme, there develops an intricate site-specific coordination between anabolic and catabolic hormones and the immune cytokines produced in the response to stress in general and inflammatory infection stress in particular. The endocrine-immune gradient incorporates the concepts of differential ligand binding in addition to simple changes in plasma concentrations of regulatory hormones as a means of enhancing tissue-specific effects of regulatory ligands. An essential component of the model mandates that the changing temporal milieu of endocrine and immune effectors be thought of as cascades of patterned responses wherein waves of cytokines and hormones are up- and downregulated in very specific induction/repression sequences. As such, adrenomedullin (AM) can play a master role in orchestrating differential regulation among tissues during inflammation because of its capacity to bind to multiple classes of receptors (Clark and Lowe, 1998) and elicit different tissue responses in specific tissue sites. In essence, AM is both a hormone and a cytokine. It can simultaneously regulate aspects of regional blood flow (e.g., mesenteric vs. coronary flow), immunological recruitment (neutrophil migration and margination vs. monocytic differentiation), and preferential nutrient use by tissues (decreased peripheral tissue glucose uptake) as demanded during the inflammatory response.

As depicted in Figure 1, not only is there a hierarchy in redirection of metabolic response by organ systems during inflammatory stress, but also a reprioritization between different components of organs within the same systems. Consistent with and expanded beyond the original Hammond model, the endocrine-immune gradient originally encompassed the totality of hormones within the insulin and somatotropic-growth hormone-insulin-like growth factor-I axes (anabolic), the glucocorticoid axis (catabolic), and the thyroid axis (modulatory and permissive). Overlaid with these modulators were the proinflammatory cytokines (TNF-α, IL-I, catabolic), antiinflammatory cytokines (IL-10 and

TGF-β), and anabolic and modulatory cytokines (IL-15, interferons, and colony-stimulating factors). In this review, we have further modified the hypothesis and included for relevance a depiction of how AM participates in the endocrine-immune gradient as represented by a relative level of tissue-specific expression during the inflammatory response to endotoxin challenge.

As an example of how tissues differentially respond to inflammation, we studied specific protein turnover responses in skeletal muscles during the inflammatory response to an end-stage muscle-encysted parasitic disease called sarcocystis. Interestingly, catabolic responses and loss of muscle mass was associated with postural muscles such as the psoas major, while muscle protein mass of locomotor muscles such as the rectus femoris was largely retained. Similarly, lipid deposits in subcutaneous fat are more likely to be mobilized than intraabdominal (kidney and pelvic area) fat as an energy source during the reduced voluntary food intake and increased caloric demand of subjects experiencing an inflammatory episode. There is logic in this differential response if one considers the reduction in voluntary food intake which accompanies the onset of many acute phase responses in disease, coupled with the need to redirect hepatic protein synthesis associated with generation of acute phase response proteins and antioxidant proteins (Kagan and Laskin, 2001; Laskin et al., 2001). Similarly, resources are needed in terms of calories to support the body fever response normally observed in infection and trauma.

Many of the responses of body tissues to an inflammatory insult are triggered and modulated by cytokines like TNF-α, which not only promote direct catabolism of muscle but facilitate it further through interactions with increased cortisol production and inhibition of protein anabolic hormone synthesis and release (Elsasser et al., 2000b). Changes in caloric demand and source are not an insignificant consideration in the response to inflammation, in that Kluger (1991) estimated that caloric expenditures of a fever-ridden body increase as a function of the respiratory quotient (Q_{10}) by 10–30% for each 1–1.5°C increase in core temperature. Similarly, in a review of experiments in which resting energy expenditure was measured as a function of health status, Goldstein and Elwyn (1989) demonstrated that the severe inflammatory response of sepsis in humans could increase energy expenditure as much as 60% with catabolism-associated nitrogen excretion increasing from a general mean of 4 g N/70 kg body weight per day to more than 10 g. At the heart of this regulation is the ability of cells to act as the integrators of the numerous factors which dictate how the cell needs to respond as a participant in the inflammatory response. Not very different from how people are called upon to change and respond during a crisis, cells of the body either will "lead, follow, or get out of the way." They will function as regulatory modulators (leaders, i.e., macrophages, adrenocortical cells), or organ support units (followers, i.e., vascular endothelium, cardiopulmonary tissues), or be consumed by catabolism (adipose and muscle tissues) or when dead or apoptotic (adipocytes, injured hepatocytes, etc.).

In the past we have documented extensive inverse relationships between a major anabolic hormone insulin-like growth factor-I (IGF-1) and the expression and increased plasma concentrations of the catabolic inflammatory initiator cytokine tumor necrosis factor-α (TNF-α) in both experimental models of endotoxemia (Elsasser et al., 1997) and parasitic infection (Elsasser et al., 1998). As will become evident in the present review, TNF-α plays a critical role in inflammation in that it represents one of the, if not the, major cytokine produced in the response to inflammatory stress. TNF-α triggers the cascade of subsequent cytokines and hormones that effect the needed changes in cell metabolism. In response to the bacterial toxin challenge (endotoxin), TNF-α concentrations increase dramatically in blood and locally in the paracrine response of select tissues. Correspondingly, and in proportion to the severity of the challenge, IGF-I plasma concentrations, as well as most anabolic (tissue mass accretion) processes, decrease for a period of several hours to days. A noted exception to this is the redirected anabolic production of acute phase response proteins in the liver as a countermeasure to the insult to aid in restoring homeostasis. In addition, there is a fundamental reversal of tissue metabolic priorities wherein those tissues that were lowest in their metabolic priority for assimilation of fat stores and muscle protein become some of the highest donors of these nutrient energy and amino acid resources during inflammation (Elsasser et al., 2000b). Indeed, many of catabolic responses incurred during the inflammatory response are initiated by macrophage production of TNF-α. This is especially true in selective skeletal muscle catabolism needed for liberation of glutamine to be utilized as an alternative energy source for immunologically active cells during infection (Calder and Yaqoob, 1999; Biolo et al., 1997)

TNF-α–AM–NO CONNECTION IN INFLAMMATION

Most relevant to the topic at hand is the tight relationship between proinflammatory cytokines like TNF-α and IL-1β and AM during the onset of systemic as well as localized tissue inflammatory response (Jougasaki and Burnett, 2000). Increases in circulating concentrations of AM are present in a variety of experimental as well as clinically significant infections and diseases. Examples of this include the increase in plasma AM after endotoxin administration (Minamino et al., 1998; Elsasser et al., 1999b) and other inflammatory diseases like sepsis (Hirata et al., 1996), systemic inflammatory response syndrome (Ueda et al., 1999), and Behcet's disease (Evereklioglu et al., 2000). The dynamic increases in TNF-α and corresponding increases in AM coupled with demonstrated properties of AM to decrease TNF-α and other inflammatory cytokine productions leads to the novel idea that AM and the proinflammatory cytokines form a functional feedback loop necessary to trigger the needed response to combat invading microbes and also to initiate needed recovery and cell salvage processes, to the elaboration of the potent inflammatory response mediators like nitric oxide, hydroxy radicals, peroxides, and superoxide anion which, when present in an untimely fashion or in excess, cause cell death and participate in multiple organ failure. It is most noteworthy that AM's role in the inflammatory process changes by location (cell type) and time after onset of inflammatory challenge.

In its relationship with the proinflammatory cytokine network, where TNF-α increases AM production in macrophage-monocytes, AM also suppresses IL-1β-induced TNF production in Swiss 3T3 cells (Isumi et al., 1999; Kappas et al., 2000). The interplay between AM and proinflammatory cytokine mediators is intricate. For example, while the expression of some cytokines is blunted by AM, the secretion of others such as IL-6 (Isumi et al., 1998) is increased in some cell types more than 5-fold. In addition to directing the secretory responses of immune cells in the inflammatory response, there is new evidence that AM may play a role in increasing the numbers of immune calls capable of responding to an immunological challenge by increasing the rate of differentiation of specific cell types (Kubo et al., 1998a).

DEFINING INFLAMMATION

Described in a historical account of the study of inflammation (http://aeb.cvm.okstate.edu/vmed5264), writers as far back as the first century described the primary signs of inflammation in terms of swelling, pain, redness, and heat. Subsequently, during the 19th and 20th centuries researchers such as Virchow, Conheim, Metchnikoff, and Ehrlich began to fill in the cellular and biochemical basis for these observations which today are well recognized to encompass many if not all of the possible actions of the AM peptide. These include: regional changes in blood flow, modulation of monocyte and immune cell function, electrolyte balance, modulation of hormones that affect metabolism, differential regulation of the complement cascade, stabilization of DNA (antiapoptotic factor), antimicrobial effects, and changes in cardiac performance. The inflammatory response should be thought of as a survival mechanism in which there occurs a rapid reprioritization of biological needs and systems to detect the insult, somehow "measure" the magnitude of the insult, and finally eliminate the offending character to restore balance between systems. It must be remembered that inflammation of tissues and organs during this response is caused by the body's own biochemical mediators and responses and not the trauma, pathogen, or toxin itself. For example, production of superoxide anion or NO during the inflammatory response of neutrophils, macrophages (or, in extreme situations, endothelial and epithelial tissues) to infecting microorganisms is necessary for these cells to kill or incapacitate the invading organism; overproduction of these effectors can be harmful to the body in terms of free radical damage (Levonen et al., 2001). As such, the progressive infiltration by monocytes and neutrophils at the site of insult is a key feature of inflammation and necessary for the process of host defense. However, this infiltrating process carries with it the potential consequence for organ damage when NO responses are of sufficient magnitude to promote reactive nitrogen metabolite activation. AM, acting primarily in conjunction with NO-mediated increases in localized blood flow, can sometimes contribute to a portion of the neutrophil-based damage. For example, peripherally administered AM caused a dose-dependent conjunctival hyperemia in the rabbit. An increase in inflammatory cell number and prostaglandin E(2) concentration in the aqueous humor was consistent with the increased blood flow and vascular permeability. The inflammatory effect of AM was abolished by pretreatment with the inhibitor of nitric oxide synthase, N(G)-nitro-L-arginine methylester or AM receptor antagonist, AM-(22-52).

These aberrant biochemical oxidation, peroxidation, and nitration reactions cause disruption of numerous processes vital to cell function, including mitochondrial energetics (electron transport chain, induced apoptosis), ion gradients (cell membrane potential), and protein and nucleic acid function (enzyme inactivation and apoptosis, respectively) in target and adjacent cells in a tissue or organ (Zhao et al., 2001; Shi, 2001). However, data also demonstrate that if the production of these chemical mediators is too severely disrupted, the consequences to the host can fatal! These fatal interactions apparently involve aspects of bacterial species and the specificity and selectivity of the arginine substrate inhibitor. For example, where experiments were performed during which NO production was competitively inhibited with the administration of nonmetabolizable arginine substrate analogs, experimental infection with a pathogen resulted in the inability for the defending immune cells to mount the needed NO response to kill the pathogens (Chan et al., 1995; Leib et al., 1996). Thus, stringent checks and balances need to be in place to coordinate and stabilize these biochemical events and it appears that AM performs such a coordinating function.

TRILOGY OF HOMEOSTASIS

Figure 2 depicts a relationship in which homeostasis is maintained by and reflected in the interaction between the components of immune surveillance, the regulation of tissue functional state by the endocrine system, and nutrient and metabolic status in the body.

In this figure, homeostasis is represented inside the triangle as an integrated process coordinated through the regulatory feedback loops of the endocrine and immune systems and further modified by the prevailing metabolic state. In this depiction, elements of the nervous system which might participate in some aspects of inflammation perhaps more related to trauma are intentionally omitted because the primary focus of this review is the associations between AM and inflammatory responses of infection and metabolic stress. Although much of the research on AM (as occurs with most newly discovered novel biological substances) has focused on specific, individual actions of AM in isolated test systems, it is apparent that AM has a broad range of biological effects in many physiological systems at the same time. AM production has influence over, or is influenced by, tissues or cells associated with each of these legs of the trilogy. For a comprehensive review of the interactions of AM with overall endocrine hormone secretion, the reader is referred to Martínez et al. (1998).

The development of conditions that provoke cellular responses culminating in inflammation, by necessity, disrupt homeostatic balance and call for the initiation of a new biological paradigm to combat the insult, redirect nutrient use, and provide a means to get the cellular response to the needed location. For example, vascular changes occur which allow circulating response cells such as macrophages and polymorphonuclear monocytes and neutrophils to migrate between endothelial cells lining blood vessels and take up residence in extravascular tissue sites. AM may partici-

pate in increasing the efficiency of this process by facilitating the maintenance of blood flow in its vasorelaxing effects as well as some newly identified properties to effect chemotaxis of infiltrating immune cells (Fernandez et al., 2000; Kamoi et al., 1995) and the migration and inhibition of migration of other vascular cells (Kohno et al., 1997, 1998; Horio et al., 1995).

In attempting to deliver the needed response elements to the sites of inflammation, the vascular system plays a vital role but is itself a target for many of the pathological sequellae of infection and inflammation. In the worst-case scenario, many of the causes for multiple organ failure of sepsis originate in conjunction with the differential organ vascular tension, poor tissue delivery of nutrients and oxygen and the removal of metabolic waste products of accelerated metabolism, and localized tissue hypoxia, free radical production, tissue infarct, and the onset of irreversible hypotensive shock. AM appears to play an important role in maintaining perfusion of vital organs during inflammation through its vasodilatory effects on blood vessels (Koo et al., 2000a, 2000b). Endotoxin-induced hypotension appears to be mediated through a specific AM receptor sensitive to the AM peptide sequence residues 22–52 (Mazzocchi et al., 2000). In fact, when AM is experimentally overexpressed in the vasculature of AM-transgenic mice there is a resistance to endotoxin-induced shock (Shindo et al., 2000). A clearer picture of the mechanism through which AM participated in the stress response affected by hypoxia is presented in the recent work by Garayoa et al. (2000), in which a part of the increased expression of AM during hypoxia stress was attributable to a process driven by a hypoxia induction factor called HIF-1. Where HIF-1 gene knockout animals were subjected to hypoxic condition, the induction of AM was significantly blunted in comparison to the response observed in HIF-1-intact animals.

Signals from each of these components impinge collectively at or in cells and are integrated within the cell to formulate a given output by that cell. Factors critical to how cells integrate impinging signals are: 1) changing concentrations of hormones and cytokines that reach cells from remote (endocrine-type regulation) and adjacent cells and organ structures (paracrine and autocrine modes of effector presentation); 2) the temporal character of how these concentration changes are presented to the cells (concentration $\times$ blood flow: flux); 3) the presence of receptors for the effector ligands on and within cells; 4) blood flow, nutrient and oxygen presentation to tissues and cells; 5) alterations in intracellular signal transduction interactions (cAMP, Ca^{++}, kinase phosphorylation activation, phosphodiesterase activity, RMP proteins, etc.); 6) plasma transport binding protein modulation of cell effector availability; 7) endothelial permeability; 8) mitochondrial energetics functionality; 9) rates of effector degradation and metabolic clearance; and 10) regulation of gene transcription and translation and ribosome stability and intracellular cytoskeletal-dependant resource shuttling.

TEN POTENTIAL POINTS OF AM IMPACT ON THE INFLAMMATORY PROCESS

Figure 3 represents the development of an integrated model to place into perspective the relative contributions of AM-related actions as well as factors that regulate AM in the development and remission of the inflammatory response. The numbers within the circles represent some general as well as specific influences of or on AM in the body.

Area 1

Circulation plays a prominent role in all of the considerations that shape the clinical outcome of an inflammatory episode. This is especially true in situations involving generalized states of systemic infection, and most particularly evident in those conditions that progress into shock and multiple organ failure. The circulation is, by definition, the route through which "endocrine" functions of AM are delivered to cells and organs. Increase in plasma concentrations of AM are well documented in association with inflammatory and infection disease states. In addition, where experimental models of infection were used that incorporated bacterial endotoxin challenge in vivo and in vitro as well as the direct administration of macrophage response cytokines such as tumor necrosis factor-α, increases in plasma concentrations of AM as well as specific increases in tissue mRNA for AM and AM peptide have been reported. However, the exact roles of the increased concentrations of AM in the circulation during infection stress are really not well understood. What is quite apparent is that localized production of AM, in the myriad tissues that respond to or are affected by the infection/inflammatory process, significantly influence the patterns of secretion of numerous other hormones released from traditional endocrine glands such as the pituitary, adrenal, thyroid, and pancreas. These secreted hormones reach remote tissues via the circulation. Collectively, adrenocorticotropic hormone, cortisol, thyroid hormone, insulin, and glucagon, to name the hormones which to date have been associated with AM-mediated regulation (reviewed in Martínez et al., 1998), influencing tissue metabolism via the circulation in their participation in the endocrine-immune gradient. As in most good feedback systems, checks and balances exist between the many effectors of the inflammatory response. Where AM has been shown to increase the activity of the hypothalamic–pituitary–adrenal axis, the glucocortidoid endproducts have a significant inhibitory effect on further AM production (Hattori et al., 1998).

A significant point that needs to be emphasized is that the conveyance of hormone signals via the circulation has multiple dimensions which go beyond the commonly discussed and easily measured variable, plasma concentration. The more accurate descriptions of hormone conveyance in the blood are derived from flux measurements, the concentration per unit time within the established blood flow. Flux measurements are difficult to ascertain under normal conditions and even more so during the inflammatory response due to the absence of steady-state conditions and limitations often encountered in the relative sensitivity of assays to discriminate arterial–venous concentration differences. Certainly AM participates in this flux capacity in its capacity to affect blood flow through changes in vascular resistance as well as cardiac performance (Lainchbury et al., 2000; Amatyakul et al., 2000; Wang, 2000). It is important to recognize that an underlying

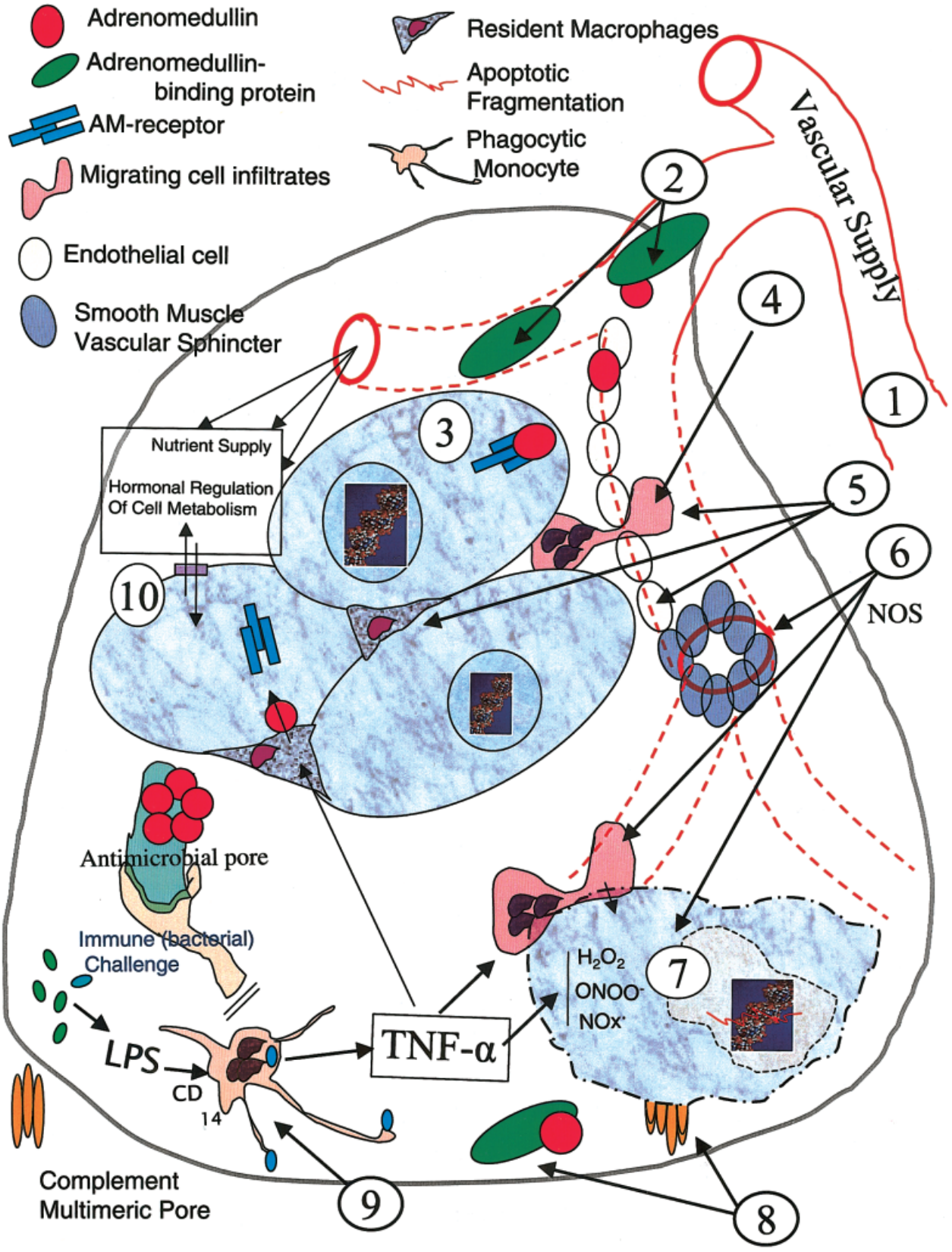

Fig. 3. Ten points or "areas" of AM impact during the inflammatory response. Please see text Areas 1–10 for details.

disease such as heart failure may alter the vasorelaxant properties of AM (Lainchbury et al., 2000) and alter the homeostatic compensating character of AM when a septic crisis erupts. Where the critical feature of a tissue response to a hormone is the availability of that hormone to a cell receptor, the presentation of that hormone to the receptor is influenced by blood flow, hormone concentration, tissue transit time, capillary and endothelial cell permeation, and the transport state of the hormone, i.e., binding protein association.

Area 2

While it is well documented that plasma AM concentrations increase in response to experimental administration of endotoxin (as a model of a sepsis effector) as well as during bacterial infection stress, it is now recognized that only a portion of the AM in the circulation is actually free AM. Elsasser et al. (1999b) characterized the presence of a 120-kD protein in the plasma of 10 animal species with specific binding capacity for AM. Further characterization of the protein revealed that the protein was complement factor H (Pio et al., 2000) and that the presence of factor H could alter the biological properties of AM. Conversely, AM bound to the AM binding protein-Factor H altered the biological functions of factor H. Plasma content of factor H was decreased in some disease states (Elsasser et al., 1999b). This implies that the delivery of AM to tissue sites during disease states is modulated in part by the transport capacity of AM binding protein-factor H as its concentrations change during the progression and remission of disease. In addition, other disease states that compromise the production of factor H will affect AM function through the differential delivery of AM to tissues, effects on transport across endothelial barriers, and metabolic clearance rate of AM.

Area 3

With AM present in the circulation and able to reach cells, the next critical feature of how AM participates in the inflammatory response is dictated by the ability of AM to bind to surface membrane receptors and effect specific sequences of hormone signal transduction. Receptors for AM have been identified in many, if not most, tissues studied and there appear to be several possible receptor interactions which can take place which affect what AM does at that particular time (Clark and Lowe, 1998). Specific receptors for AM with nanomolar binding affinities have been characterized but secondary binding capacity for AM is present in the receptor for calcitonin gene-related peptide (CGRP). The binding capacity for AM to these different receptors and the nature of the signal transduction is well reviewed elsewhere (Clark and Lowe, 1998). Important to the discussion of the role of AM in the inflammatory process is the recognition that surface membrane receptor populations are rather dynamic and the number and affinity of receptor-ligand complexes often changes during disease states. Comparing the results of two different experiments of Clementi et al. (1999, 2000), it appears that different characteristics of AM mediation of the inflammatory process may be conferred through the type of receptor on which the AM binds. In a model of ocular inflammation (Clementi et al., 2000), a hyperemic effect of AM administration with accompanied neutrophil migration was mediated via an AM-specific (AM-22-52) receptor type. However, in another situation where peritoneal inflammation was induced via the administration of acetic acid (Clementi et al., 1999), AM administration was associated with a CGRP-receptor-mediated decrease in inflammatory response. These contrasting effects of AM in the inflammatory process further illustrate how the receptor-ligand component of the endocrine-immune gradient functions to impart differential control among tissues as a hierarchy for tissue response is established. Thus, if there occur differences in the expression and function of AM receptors during inflammation, the actions of AM will be modulated. This is at present a fertile area for research in that there are no publications that directly address changing dynamics and binding characteristics of AM receptors on cell surfaces during inflammation and sepsis.

Area 4

One of the largest sources of AM, which arises during the inflammatory process, is that which is derived from circulating immune cells such as monocytes, macrophages, and neutrophils (Zaks-Zilberman et al., 1998; Minamino et al., 1998; Kubo et al., 1998b). Attracted to specific locations by tissue release of chemoattractants, the infiltrating cells serve multiple roles in the protection of health, but the role of AM as released by these cells is mostly multifunctional. Roles of AM at this juncture include regulation of localized blood flow, alterations in further production of chemokines, modulation of cytokine and nitric oxide synthase activity, and functions as antimicrobial peptides (Allaker et al., 1999). In addition to direct killing effects of AM on particular bacteria, the interaction of AM in forming ion pores in the microbial cell wall weakens the microbes and enhances the phagocytic capabilities of neutrophils and monocytes. Furthermore, because of the new-found property of AM to modulate the bioactivity of complement factor H as factor H serves as an AM plasma binding protein (Pio et al., 2001), the range of antimicrobial peptide effects of AM may be broadened in AM's capacity to modulate the inflammatory response within the complement cascade (Scott et al., 2000).

Area 5

While the monocytes are capable of responding to factors that upregulate the inflammatory process, many other cell types are capable of localized, paracrine, and autocrine production of AM. These include epithelial and vascular endothelial cells and resident immune-function cells such as Kupffer cells and astrocytes (Takahashi et al., 2000). This localized production is very important to the localized needs of the tissues. In fact, this may be the site of AM action where the temporal character of AM function is most prominent.

Area 6

Where initial needs of tissues during a response in inflammation may be to obtain increased vascular supply, later stages of cell responses need to come into play to limit potential overproduction of effector molecules such as NO decay products, superoxide anion, and per-

oxides, which become injurious to cells. However, there is a very tight association between the presence of AM and upregulation of AM and the evolution of the NO cascade. Together, AM and NO appear to complement one another in a fine-tuning of many of the physiological processes which develop during inflammation, including regulation of blood flow and precapillary sphincter-microvascular function (Chu et al., 2000). AM and NO appear to be coordinately increased in endothelial cell, monocyte, and tissue epithelial cell responses to inflammatory stimuli. The intracellular production of NO and the resulting NO metabolism cascade may play a significant part in redirecting intracellular metabolism including changes in mitochondrial function, enzyme function, and even newly discovered antioxidant properties of NO.

Area 7

In the inflammatory process, many biochemical events are triggered which result in inappropriate and deleterious chemical modification of intracellular proteins. Chemical modification of proteins leads to altered biological activity, which can, according to the specific protein, enhance or retard biochemical activity. For example, while many protein functions are decreased such as the loss of activity of neuronal superoxide dismutase (Macmillan-Crow and Cruthirds, 2001) some protein actions are enhanced, such as the increased activity of fibrinogen (Gole et al., 2000). In the first situation, cells lose the ability to neutralize toxic superoxide anion; in the second situation, increased coagulopathy associated with enhanced clot formation results in the development of microangiopathies, perfusion failure, and increased complications within the multiorgan failure scenario. This becomes a complex issue and in several instances is almost a random occurrence for which chemical modifications occur. Usually, however, these reactions involve some distinct reactions which result in the oxidation of proteins (carbonyl-formation), protein nitration, nitrosylation, thiol nitration, tyrosine and serine nitration, lipid peroxide formation, and dityrosine formation, all of which are established by the turn of events which dictate the intracellular oxidation-reduction state and therefore the direction of decay of NO, the formation of peroxynitrite and, induction of superoxide anion (Elsasser et al., 2000a; Nakazawa et al., 2000). Recent data from Chun et al. (2000) suggests that oxidative stress increases the secretion of AM in endothelial cells.

In addition, AM has been identified as a significant apoptotic factor which contributes to the restoration of homeostatic equilibrium. Much of the apoptotic response is triggered by the binding of TNF to one of two TNF cell receptors referred to as the "death" receptor. Whereas some of AM's antiapoptotic capacity may be associated with its downregulation of TNF per se, many of its countereffects on apoptosis appear to be mediated by AM effects on the target cell itself and manifest through cyclic-GMP-responses and NO-dependent mechanisms (Sata et al., 2000).

Area 8

In the inflammatory process cells that are affected in a pathological manner are affected by one of two reactions. They either undergo apoptosis or they undergo necrosis (Oberholzer et al., 2001). A significant part of the necrotic response is associated with perturbations in some aspects of the complement cascade that result in a process described as the complement-mediated membrane attack pathway (Morgan, 1999). In this destructive process, complement factors Cb5 through C8 interact to culminate in a multimeric C9 complex intercalating into cell membranes forming ion pores, which disrupt ionic currents and induce lysosomal degranulation and autodigestion of cellular components. Of course, where there is a need to eliminate cellular debris macrophages are called in and the autodestruction sequences are maintained. While yet to be investigated, the fact that the AM binding protein is a complement cascade regulatory protein (Factor H), and AM alters the catalytic capacity of Factor H (Pio et al., 2001) enhancing the cleavage of C3B by factor I, there exists a strong potential that AM might influence the induction of this membrane attack pathway. Beyond this there appears to be a connection between cell stability and AM in disease processes. The integrity of cells may be better maintained when AM induction and production are elicited (Chini et al., 1997).

Area 9

Many aspects of the larger collective process of inflammation and inflammatory response are similar to the responses observed during sepsis or the administration of bacterial extracts such as endotoxin. A key factor in the AM-inflammatory response cycle is a feedback loop that involves binding of endotoxin to the CD-14 cell surface receptor (located on many epithelial cell types as well as monocytes, leukocytes, and neutrophils), the induction of transcription of inflammatory cytokines, TNF-α and IL-1 in particular, and the upregulation of AM (and NO-synthase activity). The upregulation of AM by TNF is interesting in that TNF is largely considered a transient response initiator whose function appears to be the initiation of a cascade of responses including increased glucocorticoid production, prostaglandin mediators, as well as a significant redirection of metabolism away from anabolism towards catabolism. The regulatory loop appears to be in part contributing to the ability of AM to downregulate much of the production of these inflammatory cytokines and limit many of these decompensating influences (Koo et al., 2000).

Some additional qualities of AM may prove to be beneficial in the body's defensive inflammatory process. When, as previously discussed, antimicrobial peptide pore formation in bacteria results in enhanced phagocytic and killing capacity of microbes by activated monocytes and macrophages (enlarged area of phagocytisis) the immune challenge is cleared more rapidly. As defined by Scott and Hancock (2000), antimicrobial peptides are generally in the range of 12–50 amino acids in length possessing a net positive charge in association with high molar content ratios of basic amino acids and strong hydrophobicity facilitating their interaction with bacterial membranes. AM fits these characteristics well and as such has the potential to be developed into a therapeutic adjunct to support antimicrobial chemotherapy in a fashion in which op-

portunities for antibiotic resistance might be decreased.

Area 10

Coming full circle, cell metabolism is significantly affected by AM both by its endocrine presentation to target cells, its paracrine evolution in situ, and through effects of AM to regulate other hormones such as insulin and glucagons. In this regard, we recently defined the pancreas as a target for disease stress and inflammatory response in terms of its shock-organ-like responses to impinging multiple immune stimuli. Stemming from Martínez's observation of effects of AM localization in the pancreas and its ability to influence glucose metabolism in vivo, we conducted experiments which demonstrated that AM and NOS upregulation responses to the combination of occult, subclinical parasitic infection combined with low-level endotoxin challenge were parallel and colocalized in pancreatic islets (Elsasser et al., 1999a). The magnitude of the plasma AM response as well as the relative degree of localized increased expression of AM and NOS-Type 2 in the islets was positively correlated to the magnitude of the TNF-α response and inversely related to the absolute plasma concentrations of insulin developing in the hypoglycemic phase of the response to the endotoxin challenge. The interesting feature of this observation was that the AM-NOS responses in the pancreas persisted long after similar responses in the lung had disappeared. The observations support the concept that AM plays a key role in the progression and remission of the inflammatory response in vivo and that the role can change over time, thus influencing the observations and conclusions of investigators.

CONLUSIONS

Collectively, this overview of AM in inflammation demonstrates the vast complexity of AM bioactivity as it evolves during the upregulation and downregulation of localized and systemic inflammatory responses. In general, the more recent mass of literature on AM, AM gene responses, and associated cell responses in pathological situations suggests that AM is a survival factor and a force which functions to reestablish homeostatic accord across physiological systems. The actions of and measurable changes in AM peptide and gene responses during the inflammatory response are consistent with hypotheses and models of metabolic reprioritization, wherein the metabolic capabilities and priorities of a tissue change in accordance with the need for survival. In this regard the development of pharmacological agents with specific actions on the fate of AM and its binding protein(s) may prove valuable adjunct therapies in the remediation of inflammatory reactions.

REFERENCES

Allaker RP, Zihni C, Kapas S. 1999. An investigation into the antimicrobial effects of adrenomedullin on members of the skin, oral, respiratory tract and gut microflora. FEMS Immunol Med Microbiol 23:289–293.

Biolo G, Toigo G, Ciocchi B, Situlin R, Iscra F, Gullo A, Guarnieri G. 1997. Metabolic response to injury and sepsis: changes in protein metabolism. Nutrition 13(Suppl 9):52S–57S.

Calder PC, Yaqoob P. 1999. Glutamine and the immune system. Amino Acids 17:227–241.

Chan J, Tanaka K, Carroll D, Flynn J, Bloom BR. 1995. Effects of nitric oxide synthase inhibitors on murine infection with Mycobacterium tuberculosis. Infect Immunol 63:736–740.

Chini EN, Chini CCS, Bolliger C, Jougasaki M, Grande JP, Burnett JC, Dousa TP. 1997. Cytoprotective effects of adrenomedullin in glomerular cell injury: central role of cAMP signaling pathway. Kidney Int 52:917–925.

Chu DQ, Choy M, Foster P, Cao T, Brain SD. 2000. A comparative study of the ability of calcitonin gene-related peptide and adrenomedullin 13-52 to modulate microvascular but not thermal hyperalgesia responses. Br J Pharm 130:1589–1596.

Chun T-H, Itoh H, Saito T, Yamahara K, Doi K, Mori Y, Ogawa Y, Yamashita J, Tanaka T, Inoue M, Masatsugu K, Sawada N, Fukunaga Y, Nakao K. 2000. Oxidative stress augments secretion of endothelium-derived relaxing peptides, C-type natriuretic peptide and adrenomedullin. J Hypertens 18:575–580.

Clark AJL, Lowe S. 1998. Adrenomedullin receptors and their genes: a family in evolution? In: Martínez A, Cuttitta F, editors. Adrenomedullin. Washington, DC: IOS Press. p 41–48.

Clementi G, Caruso A, Cutuli VMC, Practori A, Mangano NG, Amico-Roxas M. 1999. Antiinflammatory activity of adrenomedullin in the acetic acid peritonitis of rats. Life Sci (Pharm Lett) 65:PL203–208.

Clementi G, Floriddia ML, Prato A, Marino A, Drago F. 2000. Adrenomedullin and ocular inflammation in the rabbit. Eur J Pharmacol 400:321–326.

Elsasser TH, Kahl S, Steele NC, Rumsey TS. 1997. Nutritional modulation of somatotropic axis-cytokine relationships in cattle: a brief review. Comp Biochem Physiol 116:209–221.

Elsasser TH, Sartin JL, McMahon C, Romo G, Fayer R, Kahl S, Blagburn B. 1998. Changes in somatotropic axis response and body composition during growth hormone administration in progressive cachectic parasitism. Domest Anim Endocrinol 15:239–255.

Elsasser TH, Sartin JL, Martínez A, Kahl S, Montuenga L, Pio R, Fayer R, Miller M, Cuttitta F. 1999a. Underlying disease stress augments plasma and tissue adrenomedullin (AM) responses to endotoxin: colocalized increases in AM and inducible nitric oxide synthase within pancreatic islets. Endocrinology 140:5402–5411.

Elsasser TH, Kahl S, Martínez A, Montuenga LM, Pio R, Cuttitta F. 1999b. Adrenomedullin binding protein in the plasma of multiple species: characterization by radioligand blotting. Endocrinology 140:4908–4911.

Elsasser TH, Kahl S, Rumsey TS, Blum JW. 2000a. Modulation of growth performance in disease: reactive nitrogen compounds and their impact on cell proteins. Domest Anim Endocrinol 19:75–84.

Elsasser TH, Klasing KC, Filipov N, Thompson F. 2000b. The metabolic consequences of stress: targets for stress and priorities of nutrient use. In: Moberg GP, Mench JA, editors. The biology of animal stress. New York: CAB International. p 77–110.

Evereklioglu C, Yurekli M, Er H, Ozbec E, Hazneci E, Cekmen M, Inaloz HS. 2000. Increased plasma adrenomedullin levels in patients with Behcet's disease. Dermatology 201:312–315.

Fernandez S, Knopf MA, McGillis JP. 2000. Calcitonin-gene related peptide (CGRP) inhibits interleukin-7-induced pre-B cell colony formation. J Leuk Biol 67:669–676.

Garayoa M, Martìnez A, Lee S, Pio R, An WG, Neckers L, Trepel J, Montuenga LM, Ryan H, Johnson R, Gassmann M, Cuttitta F. 2000. Hypoxia-inducible factor-1 (HIF-1) up-regulates adrenomedullin expression in human tumor cell lines during oxygen deprivation: a possible promotion mechanism of carcinogenesis. Mol Endocrinol 2000 14:848–862.

Goldstein SA, Elwyn DH. 1989. The effects of injury and sepsis on fuel utilization. Annu Rev Nutrit 9:445–473.

Gole MD, Souza JM, Choi I, Hertkorn C, Malcolm S, Foust RF 3rd, Finkel B, Lanken PN, Ischiropoulos H. 2000. Plasma proteins modified by tyrosine nitration in acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol 278:L961–967.

Hammond J. 1952. Physiological limits to intensive production in animals. Br Agric Bull 2:8–12.

Hattori Y, Murakami Y, Atsuta H, Minamino N, Kangawa K, Kasai K. 1998. Glucacorticoid regulation of adrenomedullin in a rat model of endotoxic shock. Life Sci 62:181–189.

Hinson JP, Kappas S. 1998. The role of endothelial cell products in the regulation of adrenocortical function: actions of endothelin, nitric oxide, adrenomedullin and PAMP. Horm Metab Res 30:334–340.

Hirata Y, Mitaka C, Sato K, Nagura T, Tsunoda Y, Amaha K, Marumo F. 1996. Increased circulating adrenomedullin, a novel vasodilatory peptide, in sepsis. J Clin Endo Metab 81:1449–1453.

Horio T, Kohono M, Kano H, Ikeda M, Yasunari K, Yokokawa K, Minami M, Takeda T. 1995. Adrenomedullin as a novel antimigration factor of vascular smooth muscle cells. Circ Res 77:660–664.

Isumi Y, Minamino N, Kubo A, Nishimoto N, Yoshizaki K, Yoshioka M, Kangawa K, Matsuo H. 1998. Adrenomedullin stimulates interleukin-6 production in Swiss 3T3 cells. Biochem Biophys Res Commun 244:325–331.

Isumi Y, Kubo A, Katafuchi T, Kangawa K, Minamino N. 1999. Adrenomedullin suppresses interleukin-1β-induced tumor necrosis factor-α production in Swiss 3T3 cells. FEBS Lett 463:110–114.

Jougasaki M, Burnett JC. 2000. Adrenomedullin: potential in physiology and pathophysiology. Life Sci 66:855–872.

Kagan VE, Laskin JD. 2001. Direct and indirect antioxidant effects of nitric oxide: radically unsettled issues. Antioxid Redox Signal 3:173–176.

Kamoi H, Kanazawa H, Hirata K, Kurihara N, Yano Y, Otani S. 1995. Adrenomedullin inhibits the secretion of cytokine-induced neutrophil chemoattractant, a member of the interleukin-8 family, from rat alveolar macrophages. Biochem Biophys Res Commun 211:1031–1035.

Kappas S, Hagi-Pavli E, Ajmal I, Farthing P. 2000. Role of adrenomedullin in the inflammatory response. Proc 2nd Int Symp Adrenomedullin PAMP Abstract 0–24.

Kluger M. 1991. Fever: the role of pyrogens and cryogens. Physiol Rev 71:93–108.

Kohno M, Yokokawa K, Kano H, Yasunari K, Minami M, Hanehira T, Yoshikawa J. 1997. Adrenomedullin is a potent inhibitor of angiotensin II-induced migration of human coronary artery smooth muscle cells. Hypertension 29:1309–1313.

Kohno M, Yokokawa K, Yasunari K, Minami M, Kano H, Hanehira T, Yoshikawa J. 1998. Induction by lysophosphatidylcholine, a major phospholipid component of athrogenic lipoproteins, of human coronary artery smooth muscle cell migration. Circulation 98:353–359.

Koo DJ, Yoo P, Cioffi BA, Bland KI, Chaudry IH, Wang, P. 2000a. Mechanism of the beneficial effects of pentoxyfylline during sepsis: maintenance of adrenomedullin responsiveness and downregulation of inflammatory cytokines. J Surg Res 91:70–76.

Koo DJ, Zhou M, Chaudry IH, Wang P. 2000b. The role of adrenomedullin in producing differential hemodynamic responses during sepsis. J Surg Res 95:207–218.

Kubo A, Minamino N, Iaumi Y, Kangawa K, Dohi K, Matsuo H. 1998a. Adrenomedullin production is correlated with differentiation in human leukemia cell lines and peripheral blood monocytes. FEBS Lett 426:233–237.

Kubo A, Minamino N, Isumi Y, Katafuchi T, Kangawa K, Dohi K, Matsuo H. 1998b. Production of adrenomedullin in macrophage cell line and peritoneal macrophage. J Biol Chem 273:16730–16738.

Lainchbury JG, Meyer DM, Jougasaki M, Burnett Jr JC, Redfield MM. 2000. Effects of adrenomedullin on load and myocardial performance in normal and heart-failure dogs. Am J Physiol Heart Circ Physiol 279:H1000–1006.

Laskin JD, Heck DE, Gardner CR, Laskin DL. 2001. Prooxidant and antioxidant functions of nitric oxide in liver toxicity. Antioxid Redox Signal 3:261–272.

Leib SL, Kim YS, Black SM, Ferriero DM, Tauber MG. 1996. Detrimental effect of nitric oxide inhibition in experimental bacterial meningitis. Ann Neurol 39:555–556.

Levonen A, Patel RP, Brookes P, Go Y-M, Jo H, Parthasarathy S, Anderson PG, Darley-Usmar VM. 2001. Mechanisms of cell signaling by nitric oxide and peroxynitrite: from mitochondria to MAO kinase. Antioxid Redox Signal 3:215–229.

Levy O. 1996. Antibiotic proteins of polymorphonuclear leukocytes. Eur J Haematol 56:263–277.

Martinez A, Weaver C, Lopez J, Bhathena SJ, Elsasser TH, Miller MJ, Moody TW, Unsworth EJ, Cuttitta F. 1996. Regulation of insulin secretion and blood glucose metabolism by adrenomedullin. Endocrinology 137:2626–2632.

Martínez A, Miller M-J, Montuenga LM, Cuttitta F. 1998. Adrenomedullin regulates hormone secretion. In: Martínez A, Cuttitta F, editors. Adrenomedullin. Washington, DC: IOS Press. p 185–196.

Macmillan-Crow LA, Cruthirds DL. 2001. Invited review: manganese superoxide dismutase in disease. Free Radic Res 34:325–336.

Mazzocchi G, Albertin G, Nussdorfer GG. 2000. Adrenomedullin (ADM), acting through ADM(22-52)-sensitive receptors, is involved in the endotoxin-induced hypotension in rats. Life Sci 66:1445–1450.

Minamino N, Isumi Y, Kangawa K, Kitamura K, Matsuo H. 1998. Adrenomedullin production in vascular cells and its function in the vascular wall In: Martínez A, Cuttitta F, editors. Adrenomedullin. Washington, DC: IOS Press. p 79–102.

Morgan BP. 1999. Regulation of the complement membrane attack pathway. Crit Rev Immunol 19:173–198.

Nakazawa H, Fukuyama N, Takizawa S, Tsuji C, Yoshitake M, Ishida H. 2000. Nitrotyrosine formation and its role in various pathological conditions. Free Radic Res 33:771–784.

Oberholzer C, Oberholzer A, Clare-Salzler M, Moldawer LL. 2001. Apoptosis in sepsis: a new target for therapeutic exploration. FASEB J 15:879–892.

Pio R, Martínez A, Unsworth EJ, Kowalak JA, Bengoechea JA, Zipfel PF, Elsasser TH, Cuttitta F. 2001. Complement factor H is a serum-binding protein for adrenomedullin, and the resulting complex modulates the bioactivities of both partners. J Biol Chem 276:12292–12300.

Sata M, Kakoki M, Nagata D, Nishimatsu H, Suzuki E, Aoyagi T, Sugiura S, Kojima H, Nagano T, Kangawa K, Matsuo H, Omata M, Nagai R, Hirata Y. 2000. Adrenomedullin and nitric oxide inhibit human endothelial cell apoptosis via a cyclic GMP-mediated mechanism. Hypertension 36:83–88.

Scott M, Hancock REW. 2000. Cationic antimicrobial peptides and their multifunctional role in the immune system. Crit Rev Immunol 20:407–431.

Shi Y. 2001. A structural view of mitochondria-mediated apoptosis. Nat Struct Biol 8:394–401.

Shindo T, Kurihara H, Maemura K, Kurihara Y, Kuwaki T, Izumida T, Minamino N, Ju K, Morita H, Oh-Hashi Y, Kumada M, Kangawa K, Nagai R, Yazaki Y. 2000. Hypotension and resistance to lipopolysaccharide-induced shock in transgenic mice overexpressing adrenomedullin in their vasculature. Circulation 101:2309–2316.

Shoji H, Minamino N, Kangawa K, Matsuo H. 1995. Endotoxin markedly elevates plasma concentrations and gene transcription of adrenomedullin in the rat. Biochem Biophys Res Commun 215:531–537.

Takahashi K, Nakayama M, Totsume K, Murakami O, Sone M, Kitamuro T, Yoshinoya A, Shibahara S. 2000. Increased secretion of adrenomedullin from cultured astrocytes by cytokines. J Neurochem 74:99–103.

Ueda S, Nishio K, Minamino N, Kubo A, Akai Y, Kangawa K, Matsuo H, Fugimura Y, Yoshikoa A, Masui K, Doi N, Murao Y, Miyamoto S. 1999. Increased plasma levels of adrenomedullin in patients with systemic inflammatory response syndrome. Am J Respir Crit Care Med 160:132–136.

Wang P. 2000. The role of adrenomedullin in cardiovascular responses during sepsis. Proc 2nd Int Symp Adrenomedullin PAMP Abstract 0–21.

Zaks-Zilberman M, Salkowski CA, Elsasser T, Cuttitta F, Vogel S. 1998. Induction of adrenomedullin mRNA and protein by lipopolysaccharide and Paclitaxel (Taxol) in murine macrophages. Infect Immunol 66:4669–4675.

Zhao Y, Li S, Childs EE, Kuharsky DK, Yin XM. 2001. Activation of pro-death Bcl-2 family proteins and mitochondria apoptosis pathway in TNFα-induced liver injury. J Biol Chem 276:27432–27440.